

11-08-00

A

11/07/00 jc715 U.S. PTO	Docket Number	5000XP1	jc928 U.S. PTO 09/707589 11/07/00
	FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10		
Express Mail Label Number		EL034399689US	Date of Deposit
		November 7, 2000	

Address to: Assistant Commissioner for Patents  
Box Patent Application  
Washington, DC 20231

## UTILITY PATENT APPLICATION TRANSMITTAL AND FEE SHEET

Transmitted herewith for filing under 37 CFR §1.53(b) is the utility patent application of

Applicant (or identifier): CHRISTOPHER PERRY

Title: INBRED MAIZE LINE NP2052

Enclosed are:

1. ☒ Specification (Including Claims and Abstract) - 30 pages
2. ☐ Drawings - sheets
3. ☒ Unexecuted Declaration and Power of Attorney (original or copy)
4. ☐ Microfiche Computer Program (appendix)
5. ☐ Nucleotide and/or Amino Acid Sequence Submission
  - ☐ Computer Readable Copy
  - ☐ Paper Copy
  - ☐ Statement Verifying Identity of Above Copies
6. ☐ Preliminary Amendment
7. ☐ Assignment Papers (Cover Sheet & Document(s))
8. ☐ English Translation of
9. ☐ Information Disclosure Statement
10. ☐ Certified Copy of Priority Document(s)
11. ☒ Return Receipt Postcard
12. ☒ Other: Bibliographic Data Sheet

Filing fee calculation:

- ☐ Before calculating the filing fee, please enter the enclosed Preliminary Amendment.
- ☐ Before calculating the filing fee, please cancel claims

Basic Filing Fee								\$	710
Multiple Dependent Claim Fee (\$ 270)								\$	
Foreign Language Surcharge (\$ 130)								\$	
	For	Number Filed		Number Extra		Rate			
Extra Claims	Total Claims	42	-20	22	x	\$	18	=	\$ 396
	Independent Claims	3	-3	0	x	\$	80	=	\$
TOTAL FILING FEE								\$	1,106

- ☒ Please charge Deposit Account No. 19-0134 in the name of Novartis Corporation in the amount of \$1,106. An additional copy of this paper is enclosed. The Commissioner is hereby authorized to charge any additional fees under 37 CFR §1.16 and §1.17 which may be required in connection with this application, or credit any overpayment, to Deposit Account No. 19-0134 in the name of Novartis Corporation.

Please address all correspondence to the address associated with Customer No. 022847, which is currently:

Larry W. Stults  
Novartis Agribusiness Biotechnology Research Inc.  
Patent Department  
P.O. Box 12257  
Research Triangle Park, NC 27709-2257

Please direct all telephone calls to the undersigned at the number given below, and all telefaxes to (919) 541-8689.

Respectfully submitted,



Date: November 7, 2000

Bruce Vrana  
Attorney for Applicant  
Reg. No. 38,672  
Tel. No. (919) 541-8614

INVENTOR INFORMATION

Inventor One Given Name:: Christopher  
Family Name:: Perry  
Postal Address Line One:: 731 Birch Lane  
City:: Northfield  
State or Province:: Minnesota  
Country:: USA  
Postal or Zip Code:: 55057  
City of Residence:: Northfield  
State or Province of Residence:: Minnesota  
Country of Residence:: USA  
Citizenship Country:: US



CORRESPONDENCE INFORMATION

Correspondence Customer Number:: 022847  
Fax One:: 919 541-8689  
Electronic Mail One:: bruce.vrana@nabri.novartis.com

APPLICATION INFORMATION

Title Line One:: INBRED MAIZE LINE NP2052  
Total Drawing Sheets:: 0  
Formal Drawings?: No  
Application Type:: Utility  
Docket Number:: 5000XP1  
Secrecy Order in Parent Appl.?: No

Source:: PrintEFS Version 1.0.1

00207 68520260

## INBRED MAIZE LINE NP2052

### FIELD OF THE INVENTION

This invention is in the field of maize breeding, specifically relating to an inbred maize line designated NP2052.

### BACKGROUND OF THE INVENTION

The goal of plant breeding is to combine in a single variety or hybrid various desirable traits. For field crops, these traits may include resistance to diseases and insects, tolerance to heat and drought, reducing the time to crop maturity, greater yield, and better agronomic quality. With mechanical harvesting of many crops, uniformity of plant characteristics such as germination and stand establishment, growth rate, maturity, and plant and ear height, is important.

Field crops are bred through techniques that take advantage of the plant's method of pollination. A plant is self-pollinated if pollen from one flower is transferred to the same or another flower of the same plant. A plant is cross-pollinated if the pollen comes from a flower on a different plant. Plants that have been self-pollinated and selected for type for many generations become homozygous at almost all gene loci and produce a uniform population of true breeding progeny. A cross between two different homozygous lines produces a uniform population of hybrid plants that may be heterozygous for many gene loci. A cross of two plants each heterozygous at a number of gene loci will produce a population of hybrid plants that differ genetically and will not be uniform.

Maize (*Zea mays* L.), often referred to as corn in the United States, can be bred by both self-pollination and cross-pollination techniques. Maize has separate male and female flowers on the same plant, located on the tassel and the ear, respectively. Natural pollination occurs in maize when wind blows pollen from the tassels to the silks that protrude from the tops of the ears.

A reliable method of controlling male fertility in plants offers the opportunity for improved plant breeding. This is especially true for development of maize hybrids, which relies upon some sort of male sterility system. There are several options for controlling male fertility available to breeders, such as: manual or mechanical emasculation (or detasseling), cytoplasmic male sterility, genetic male sterility, gametocides and the like.

Hybrid maize seed is typically produced by a male sterility system incorporating manual or mechanical detasseling. Alternate strips of two maize inbreds are planted in a field, and the pollen-bearing tassels are removed from one of the inbreds (female). Providing that there is sufficient

The laborious, and occasionally unreliable, detasseling process can be avoided by using cytoplasmic male-sterile (CMS) inbreds. Plants of a CMS inbred are male sterile as a result of factors resulting from the cytoplasmic, as opposed to the nuclear, genome. Thus, this characteristic is inherited exclusively through the female parent in maize plants, since only the female provides cytoplasm to the fertilized seed. CMS plants are fertilized with pollen from another inbred that is not male-sterile. Pollen from the second inbred may or may not contribute genes that make the hybrid plants male-fertile. Seed from detasseled fertile maize and CMS produced seed of the same hybrid can be blended to insure that adequate pollen loads are available for fertilization when the hybrid plants are grown.

Another system useful in controlling male sterility makes use of gametocides. Gametocides are not a genetic system, but rather a topical application of chemicals. These chemicals affect cells that are critical to male fertility. The application of these chemicals affects fertility in the plants only for the growing season in which the gametocide is applied (see Carlson, Glenn R., U.S. Pat. No. 4,936,904, which is incorporated herein by reference). Application of the gametocide, timing of the application and genotype specificity often limit the usefulness of the approach.

2

are evaluated to determine which of those have commercial potential. Plant breeding and hybrid development are expensive and time-consuming processes.

Pedigree breeding starts with the crossing of two genotypes, each of which may have one or more desirable characteristics that is lacking in the other or which complements the other. If the two original parents do not provide all the desired characteristics, other sources can be included in the breeding population. In the pedigree method, superior plants are selfed and selected in successive generations. In the succeeding generations the heterozygous condition gives way to homogeneous lines as a result of self-pollination and selection. Typically in the pedigree method of breeding five or more generations of selfing and selection is practiced: F1 to F2; F3 to F4; F4 to F5, etc.

A single cross maize hybrid results from the cross of two inbred lines, each of which has a genotype that complements the genotype of the other. The hybrid progeny of the first generation is designated F1. In the development of commercial hybrids only the F1 hybrid plants are sought. Preferred F1 hybrids are more vigorous than their inbred parents. This hybrid vigor, or heterosis, can be manifested in many polygenic traits, including increased vegetative growth and increased yield.

The development of a maize hybrid involves three steps: (1) the selection of plants from various germplasm pools for initial breeding crosses; (2) the selfing of the selected plants from the breeding crosses for several generations to produce a series of inbred lines, which, although different from each other, breed true and are highly uniform; and (3) crossing the selected inbred lines with different inbred lines to produce the hybrid progeny (F1). During the inbreeding process in maize, the vigor of the lines decreases. Vigor is restored when two different inbred lines are crossed to produce the hybrid progeny (F1). An important consequence of the homozygosity and homogeneity of the inbred lines is that the hybrid between a defined pair of inbreds will always be the same. Once the inbreds that give a superior hybrid have been identified, the hybrid seed can be reproduced indefinitely as long as the homogeneity of the inbred parents is maintained.

A single cross hybrid is produced when two inbred lines are crossed to produce the F1 progeny. A double cross hybrid is produced from four inbred lines crossed in pairs ( $A \times B$  and  $C \times D$ ) and then the two F1 hybrids are crossed again  $(A \times B) \times (C \times D)$ . Much of the hybrid vigor exhibited by F1 hybrids is lost in the next generation (F2). Consequently, seed from hybrids is not used for planting stock.

Hybrid seed production requires elimination or inactivation of pollen produced by the female parent. Incomplete removal or inactivation of the pollen provides the potential for self-pollination. This inadvertently self-pollinated seed may be unintentionally harvested and packaged with hybrid seed. Once the seed is planted, it is possible to identify and select these self-pollinated plants. These self-pollinated plants will be genetically equivalent to the female inbred line used to produce the

hybrid. Typically these self-pollinated plants can be identified and selected due to their decreased vigor. Female selfs are identified by their less vigorous appearance for vegetative and/or reproductive characteristics, including shorter plant height, small ear size, ear and kernel shape, cob color, or other characteristics.

Identification of these self-pollinated lines can also be accomplished through molecular marker analyses. See, "The Identification of Female Selfs in Hybrid Maize: A Comparison Using Electrophoresis and Morphology", Smith, J. S. C. and Wych, R. D., *Seed Science and Technology* 14, pp. 1-8 (1995), the disclosure of which is expressly incorporated herein by reference. Through these technologies, the homozygosity of the self-pollinated line can be verified by analyzing allelic composition at various loci along the genome. Those methods allow for rapid identification of the invention disclosed herein. See also, "Identification of Atypical Plants in Hybrid Maize Seed by Postcontrol and Electrophoresis" Sarca, V. et al., *Probleme de Genetica Teoritca si Aplicata* Vol. 20 (1) p. 29-42.

As is readily apparent to one skilled in the art, the foregoing are only two of the various ways by which the inbred can be obtained by those looking to use the germplasm. Other means are available, and the above examples are illustrative only.

Maize is an important and valuable field crop. Thus, a continuing goal of plant breeders is to develop high-yielding maize hybrids that are agronomically sound based on stable inbred lines. The reasons for this goal are obvious: to maximize the amount of grain produced with the inputs used and minimize susceptibility of the crop to pests and environmental stresses. To accomplish this goal, the maize breeder must select and develop superior inbred parental lines for producing hybrids. This requires identification and selection of genetically unique individuals that occur in a segregating population. The segregating population is the result of a combination of crossover events plus the independent assortment of specific combinations of alleles at many gene loci that results in specific genotypes. The probability of selecting any one individual with a specific genotype from a breeding cross is infinitesimal due to the large number of segregating genes and the unlimited recombinations of these genes, some of which may be closely linked. However, the genetic variation among individual progeny of a breeding cross allows for the identification of rare and valuable new genotypes. These new genotypes are neither predictable nor incremental in value, but rather the result of manifested genetic variation combined with selection methods, environments and the actions of the breeder. Thus, even if the entire genotypes of the parents of the breeding cross were characterized and a desired genotype known, only a few, if any, individuals having the desired genotype may be found in a large segregating F2 population. Typically, however, neither the genotypes of the breeding cross parents nor the desired genotype to be selected is known in any detail. In addition, it is not

known how the desired genotype would react with the environment. This genotype by environment interaction is an important, yet unpredictable, factor in plant breeding. A breeder of ordinary skill in the art cannot predict the genotype, how that genotype will interact with various climatic conditions or the resulting phenotypes of the developing lines, except perhaps in a very broad and general fashion. A breeder of ordinary skill in the art would also be unable to recreate the same line twice from the very same original parents, as the breeder is unable to direct how the genomes combine or how they will interact with the environmental conditions. This unpredictability results in the expenditure of large amounts of research resources in the development of a superior new maize inbred line.

### SUMMARY OF THE INVENTION

According to the invention, there is provided a novel inbred maize line, designated NP2052. This invention thus relates to the seeds of inbred maize line NP2052, to the plants of inbred maize line NP2052, and to methods for producing a maize plant by crossing the inbred line NP2052 with itself or another maize line. This invention further relates to hybrid maize seeds and plants produced by crossing the inbred line NP2052 with another maize line.

The invention is also directed to inbred maize line NP2052 into which one or more specific, single gene traits, for example transgenes, have been introgressed from another maize line. Preferably, the resulting line has essentially all of the morphological and physiological characteristics of inbred maize line of NP2052, in addition to the one or more specific, single gene traits introgressed into the inbred, preferably the resulting line has all of the morphological and physiological characteristics of inbred maize line of NP2052, in addition to the one or more specific, single gene traits introgressed into the inbred. The invention also relates to seeds of an inbred maize line NP2052 into which one or more specific, single gene traits have been introgressed and to plants of an inbred maize line NP2052 into which one or more specific, single gene traits have been introgressed. The invention further relates to methods for producing a maize plant by crossing plants of an inbred maize line NP2052 into which one or more specific, single gene traits have been introgressed with themselves or with another maize line. The invention also further relates to hybrid maize seeds and plants produced by crossing plants of an inbred maize line NP2052 into which one or more specific, single gene traits have been introgressed with another maize line.



## DEFINITIONS

In the description and examples that follow, a number of terms are used herein. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided. Below are the descriptors used in the data tables included herein. All linear measurements are in centimeters unless otherwise noted.

Heat units	$(\text{Max Temp}(\leq 86 \text{ deg. F.}) + \text{Min Temp}(\geq 50 \text{ deg. F.})) / 2 - 50$
EMRGN	Final number of plants per plot
KRTP	Kernel type: 1.sweet 2.dent 3.flint 4.flour 5.pop 6.ornamental 7.pipcorn 8.other
ERTLP	% Root lodging (before anthesis)
GRNSP	% Brittle snapping (before anthesis)
TBANN	Tassel branch angle of 2nd primary lateral branch (at anthesis)
LSPUR	Leaf sheath pubescence of second leaf above the ear (at anthesis) 1-9 (1=none)
ANGBN	Angle between stalk and 2nd leaf above the ear (at anthesis)
CR2L	Color of 2nd leaf above the ear (at anthesis)
GLCR	Glume Color
GLCB	Glume color bars perpendicular to their veins (glume bands): 1.absent 2.present
ANTC	Anther color
PLQUR	Pollen Shed: 0-9 (0=male sterile)
HU1PN	Heat units to 10% pollen shed
HUPSN	Heat units to 50% pollen shed
SLKC	Silk color
HU5SN	Heat units to 50% silk
SLK5N	Days to 50% silk in adapted zone
HU9PN	Heat units to 90% pollen shed
HUPLN	Heat units from 10% to 90% pollen shed
DA19	Days from 10% to 90% pollen shed
LAERN	Number of leaves above the top ear node
MLWVR	Leaf marginal waves: 1-9 (1=none)
LFLCR	Leaf longitudinal creases: 1-9 (1=none)
ERLLN	Length of ear leaf at the top ear node
ERLWN	Width of ear leaf at the top ear node at the widest point
PLHCN	Plant height to tassel tip
ERHCN	Plant height to the top ear node

LTEIN	Length of the internode between the ear node and the node above
LTASN	Length of the tassel from top leaf collar to tassel tip
LTBRN	Number of lateral tassel branches that originate from the central spike
EARNP	Number of ears per stalk
APBRR	Anthocyanin pigment of brace roots: 1.absent 2.faint 3.moderate 4.dark
TILLN	Number of tillers per plant
HSKC	Husk color 25 days after 50% silk (fresh)
HSKD	Husk color 65 days after 50% silk (dry)
HSKTR	Husk tightness 65 days after 50% silk: 1-9 (1=loose)
HSKCR	Husk extension: 1.short (ear exposed) 2.medium (8 cm) 3.long (8-10 cm) 4.very long (>10 cm)
HEPSR	Position of ear 65 days after 50% silk: 1.upright 2.horizontal 3.pendent
STGRP	% Staygreen at maturity
DOPN	% dropped ears 65 days after anthesis
LRTRN	% root lodging 65 days after anthesis
HU25	Heat units to 25% grain moisture
HUSG	Heat units from 50% silk to 25% grain moisture in adapted zone
DSGM	Days from 50% silk to 25% grain moisture in adapted zone
SHLNN	Shank length
ERLNN	Ear length
ERDIN	Diameter of the ear at the midpoint
EWGTN	Weight of a husked ear (grams)
KRRWR	Kernel rows: 1.indistinct 2.distinct
KRNAR	Kernel row alignment: 1.straight 2.slightly curved 3.curved
ETAPR	Ear taper: 1.slight 2.average 3.extreme
KRRWN	Number of kernel rows
COBC	Cob color
COBDN	Diameter of the cob at the midpoint
KRTP	Endosperm type: 1.sweet 2.extra sweet 3.normal 4.high amylose 5.waxy 6.high protein 7.high lysine 8.super sweet 9.high oil 10.other
KRCL	Hard endosperm color
ALEC	Aleurone color
ALCP	Aleurone color pattern: 1.homozygous 2.segregating
KRLNN	Kernel length (mm)

KRWDN	Kernel width (mm)
KRDPN	Kernel thickness (mm)
K100N	100 kernel weight (grams)
KRPRN	% round kernels on 13/64 slotted screen
GRLSR	Grey leaf spot severity rating; 1=resistent, 9=susceptible.
INTLR	Intactness rating of plants at time of harvest; 1=all foliage intact, 9=all plants broken below the ear.
LRTLPL	Percentage of plants lodged, leaning >30 degrees from vertical, but unbroken at harvest.
MST_P	Percent grain moisture at harvest.
SCLBR	Southern corn leaf blight severity rating; 1=resistent, 9=susceptible.
STKLP	Percentage of plants with stalks broken below the ear at time of harvest.
YBUAN	Grain yield expressed as bushels per acre adjusted to 15.5% grain moisture.
STBWR	Stewart Bacterial Wilt
ERLNN	Ear Length
CRSTR	Common Rust Rating
GRQUR	Grain Quality
PLTAR	Plant Appearance
HUBLN	Heat Units to Black Layer
TSTWN	Test Weight in LBS/BU
PSTSP	Push Test for Stalk/Root Quality on Erect Plants
ERGRR	Early Growth:6+ Leaf Stage

#### DETAILED DESCRIPTION OF THE INVENTION

Inbred maize lines are typically developed for use in the production of hybrid maize lines. Inbred maize lines need to be highly homogeneous, homozygous and reproducible to be useful as parents of commercial hybrids. There are many analytical methods available to determine the homozygotic and phenotypic stability of these inbred lines.

The oldest and most traditional method of analysis is the observation of phenotypic traits. The data is usually collected in field experiments over the life of the maize plants to be examined. Phenotypic characteristics most often observed are for traits associated with plant morphology, ear and kernel morphology, insect and disease resistance, maturity, and yield.

In addition to phenotypic observations, the genotype of a plant can also be examined. There are many laboratory-based techniques available for the analysis, comparison and characterization of plant genotype; among these are Isozyme Electrophoresis, Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), Amplified Fragment Length Polymorphisms (AFLPs), and Simple Sequence Repeats (SSRs) which are also referred to as Microsatellites.

Some of the most widely used of these laboratory techniques are Isozyme Electrophoresis and RFLPs as discussed in Lee, M., "Inbred Lines of Maize and Their Molecular Markers," *The Maize Handbook*, (Springer-Verlag, New York, Inc. 1994, at 423-432). Isozyme Electrophoresis is a useful tool in determining genetic composition, although it has relatively low number of available markers and the low number of allelic variants among maize inbreds. RFLPs have the advantage of revealing an exceptionally high degree of allelic variation in maize and the number of available markers is almost limitless. Maize RFLP linkage maps have been rapidly constructed and widely implemented in genetic studies. One such study is described in Boppenmaier, et al., "Comparisons among strains of inbreds for RFLPs", *Maize Genetics Cooperative Newsletter*, 65:1991, pg. 90. This study used 101 RFLP markers to analyze the patterns of 2 to 3 different deposits each of five different inbred lines. The inbred lines had been selfed from 9 to 12 times before being adopted into 2 to 3 different breeding programs. It was results from these 2 to 3 different breeding programs that supplied the different deposits for analysis. These five lines were maintained in the separate breeding programs by selfing or sibbing and rogueing off-type plants for an additional one to eight generations. After the RFLP analysis was completed, it was determined the five lines showed 0-2% residual heterozygosity. Although this was a relatively small study, it can be seen using RFLPs that the lines had been highly homozygous prior to the separate strain maintenance.

The production of hybrid maize lines typically comprises planting in pollinating proximity seeds of, for example, inbred maize line NP2052 and of a different inbred parent maize plant, cultivating the seeds of inbred maize line NP2052 and of said different inbred parent maize plant into plants that bear flowers, emasculating the male flowers of inbred maize line NP2052 or the male flowers of said different inbred parent maize plant to produce an emasculated maize plant, allowing cross-pollination to occur between inbred maize line NP2052 and said different inbred parent maize plant and harvesting seeds produced on said emasculated maize plant. The harvested seed are grown to produce hybrid maize plants.

Inbred maize line NP2052 can be crossed to inbred maize lines of various heterotic group (see e.g. Hallauer et al. (1988) in Corn and Corn Improvement, Sprague et al, eds, chapter 8, pages 463-564) for the production of hybrid maize lines.

TABLE I  
VARIETY DESCRIPTION INFORMATION  
Inbred maize line NP2052 is compared to inbred A619

	INBRED NP2052			INBRED A619		
<u>MATURITY</u>	Days	Heat Units		Days	Heat Units	
From emergence to 50% of plants in silk	66	1293.3		66	1314.3	
From emergence to 50% of plants in pollen	65	1267.8		64	1251.9	
From 10% to 90% pollen shed	003	0074.3		003	0093.5	
<u>PLANT</u>		Std Dev	Sample Size		Std Dev	Sample Size
cm Plant Height (to tassel tip)	180.3	22.1	10	180.8	30.4	10
cm Ear Height (to base of top ear node)	45.1	2.7	10	47.0	13.5	10
cm Length of Top Ear Internode	9.6	2.6	10	11.6	3.7	10
Average Number of Tillers	0	0	10	0	0.0	10
Average Number of Ears per Stalk	1.2	.3	11	1.1	0.2	11
Anthocyanin of Brace Roots:	3			2		
1=Absent 2=Faint 3=Moderate 4=Dark						
<u>LEAF</u>		Std Dev	Sample Size		Std Dev	Sample Size
Cm Width of Ear Node Leaf	008.0	2.6	10	008.3	2.6	10
cm Length of Ear Node Leaf	064.8	21.8	9	060.7	19.9	9
Number of leaves above top ear	6	.4	11	5	.2	11
degrees Leaf Angle	50	12.2	11	45	16.5	11
(measure from 2nd leaf above ear at						

anthesis						
to stalk above leaf)						
Leaf Color	02	(Munsell code 5GY 4/4)		03	(Munsell code 5GY $\frac{3}{4}$ )	
Leaf Sheath Pubescence	5			2		
(Rate on scale from 1=none to 9=like peach fuzz)						
Marginal Waves	4			5		
(Rate on scale from 1=none to 9=many)						
Longitudinal Creases	5			4		
(Rate on scale from 1=none to 9=many)						
<u>TASSEL</u>						
Number of Primary Lateral Branches	7	1.5	10	8	1.9	10
Branch Angle from Central Spike	63	40.2	11	44	10.5	11
Cm Tassel Length	33.9	9.6	10	34.1	9.9	10
(from top leaf collar to tassel tip)						
Pollen Shed	7			7		
(Rate on scale from 0=male sterile to 9=heavy shed)						
Anther Color	26	(Munsell code )		05	(Munsell code 2.5GY 8/6)	
Glume Color	26	(Munsell code )		05	(Munsell code 5GY 5/8)	
Bar Glumes (Glume Bands): 1=Absent 2=Present	2			2		
<u>EAR (Unhusked Data)</u>						
Silk Color (3 days after emergence)	26	(Munsell code )		05	(Munsell code 2.5GY 8/8)	
Fresh Husk Color(25 days after 50% silking)		(Munsell code )			(Munsell code )	
Dry Husk Color (65 days after 50 % silking)		(Munsell code)			(Munsell code)	

Position of Ear at Dry Husk Stage:  
1=Upright 2=Horizontal 3=Pendent  
Husk Tightness  
(Rate on scale from 1=very loose to 9=very tight)  
Husk Extension (at harvest):  
1=Short (ears exposed) 2=Medium (<8cm)  
3=Long  
(8-10 cm beyond ear tip) 4=Very long (>10 cm)

1

1

6

5

3

2

EAR (Husked Ear Data)Std Dev    Sample  
SizeStd Dev    Sample  
Size

Cm Ear Length

14.0

2.1

8

14.8

2.0

10

mm Ear Diameter at mid-point

40.1

3.1

7

44.1

6.3

9

gm Ear Weight

81.2

17.4

6

90.1

27.8

9

Number of Kernel Rows

15

0.9

6

15

1.2

9

Kernel Rows: 1=Indistinct 2=Distinct

2

2

Row Alignment:

1

2

1=Straight 2=Slightly Curved 3=Spiral

cm Shank Length

9.0

1.1

7

8.7

1.9

90

Ear Taper: 1=Slight 2=Average

2

2

3=Extreme

KERNEL (Dried)Std Dev    Sample  
SizeStd Dev    Sample  
Size

mm Kernel Length

10.2

0.4

6

10.5

1.1

8

mm Kernel Width

8.2

.8

6

8.9

1.0

8

mm Kernel Thickness

5.4

.5

6

4.9

1.1

8

% Round Kernels (Shape Grade)

79.9

18.9

6

65.4

25.6

8

Aleurone Color Pattern:

1

1

1=Homozygous 2=Segregating

Aleurone Color

18

(Munsell code

18

(Munsell code )

		7.5YR 8/4)			
Hard Endosperm Color	08	(Munsell code	07	(Munsell code 7.5YR	
		7.5YR 6/8)		7/10)	
Endosperm Type:	3		3		
1=Sweet (su1) 2=Extra Sweet (sh2)					
3=Normal Starch					
Gm Weight per 100 Kernels (unsized sample)	31.0		31.1		
<u>COB</u>		Std Dev Sample		Std Dev Sample	
		Size		Size	
mm Cob Diameter at mid-point	27.1	1.2 8	27.9	2.3 9	
Cob Color	19	(Munsell code )	19	(Munsell code )	
<u>DISEASE RESISTANCE</u> (1=most					
susceptible to 9=most resistant)					
Eye Spot (Kabatiella zeae)	6		4		
Northern Leaf Blight	7	Mixed	3	Mixed	
		Inoc.		Inoc.	
Gray Leaf Spot	5		5		
Common Rust	7		6		
<u>INSECT RESISTANCE</u> (Rate from 1=most					
susceptible to 9=most resistant)					
European Corn Borer(Osstrinia nubilalis)	6		7		
1 <sup>st</sup> Generation (Typically Whorl Leaf					
Feeding)					
2 <sup>nd</sup> Generation Corn Borer	2		4		
<u>AGRONOMIC TRAITS</u>					
Stay Green (at 65 days after anthesis)	6		5		
(rate on scale from 1=worst to 9=excellent)					
% Dropped Ears (at 65 days after anthesis)	0		0		
% Pre-anthesis Brittle snapping	0		0		



% Pre-anthesis Root Lodging	0	0
% Post-anthesis Root Lodging (at 65 days after anthesis)	0	0
Kg/ha Yield of Inbred Per Se (at 12-13% grain moisture)	5067.0	3398.9

In interpreting the foregoing color designations, reference may be made to the Munsell Glossy Book of Color, a standard color reference. Color codes: 1. light green, 2. medium green, 3. dark green, 4. very dark green 5. green-yellow, 6. pale yellow, 7. yellow, 8. yellow-orange 9. salmon, 10. pink-orange, 11. pink 12. light red, 13. cherry red. 14. red, 15. red and white, 16. pale purple, 17. purple, 18. colorless, 19. white, 20. white capped, 21. buff, 22. tan, 23. brown, 24. bronze, 25. variegated, 26. other.

There are clear differences between the above two inbreds. For example, the inbred line NP2052 silk is green-yellow (Munsell code 2.5GY 8/8) with pale purple shaded ends 5RP 4/10) and A619 is green-yellow (Munsell code 2.5GY 8/8).

Some of the more pronounced differences between NP2052 and A619 occur in the tassel. The NP2052 tassel appearance is not as compact with tassel branches having a larger angle from the central spike giving it a “droopy” look as compared to A619. The A619 tassel is compact with upright shorter tassel branches. The NP2052 anther color is pale yellow (Munsell code 5Y 8/8) with pale purple shading (Munsell code 5RP 4/10). The anther color of A619 is green-yellow (Munsell code 2.5GY 8/6). NP2052 has a glume color of medium green (Munsell code 5GY 4/6) with pale purple coloration (Munsell code 5RP 4/10). The glume of A619 is green-yellow (Munsell code 5GY 5/8). The glume band of NP2052 has a pale purple shade. The glume band of A619 is light green.

The leaf color of A619 is darker than NP2052. The leaf color for A619 is dark green (Munsell code 5GY 3/4). The leaf color for NP2052 is medium green (Munsell code 5GY 4/4).

There are several differences in disease and insect ratings when comparing NP2052 and A619. NP2052 has a rating of 7 for Northern Leaf Blight – Mixed Inoculum and A619 is rated a 3. The Eyespot rating for NP2052 is a 6 as compared to a rating of 4 for A619. The Common Rust rating for NP2052 is 7 and A619 is a 6. The First Brood Corn Borer rating for NP2052 is a 6 with A619 rated as a 7. The Second Brood Corn Borer rating for NP2052 is a 2 as compared to a 4 for A619.

The yield of NP2052 is greater than A619. The Kg/ha yield of NP2052 is 5067.0. The yield of A619 is 3398.9 Kg/ha.

Inbred maize line NP2052 is a yellow dent maize inbred that can be used as a male or female in crosses for producing first generation F1 maize hybrids. Inbred maize line NP2052 is best adapted

to the Northern Cornbelt regions of Canada and the United States. Hybrids made with NP2052 consistently yielded above trial mean in 1998 and 1999. The inbred can be used to produce hybrids from approximately 85-105 relative maturity based on the Comparative Relative Maturity Rating System for harvest moisture of grain. Inbred maize line NP2052 demonstrates reliable late season plant health and average pollen shed, with 7-8 lateral branches. In hybrid combinations, NP2052 demonstrates high yield for its maturity, sound seedling vigor, average plant stature, improved stalk strength, rapid drydown, and has an attractive plant appearance. In hybrid combination, NP2052 also demonstrates acceptable resistance to various stalk rots, good resistance to Northern Leaf Blight, good resistance to Eyespot, good resistance to Common Rust, and good resistance to First Brood Corn Borer. For its area of adaptation, NP2052 demonstrates high yields, resistance to stalk diseases, acceptable late season intactness, and reliable seedling vigor.

#### Origin and Breeding History of Corn Inbred Line NP2052

Inbred corn line NP2052 was derived from self pollinating the hybrid Pioneer Brand 3751. After development of the  $S_0$  (or  $F_2$ ) population, the breeding method was simple pedigree ear-to-row.

The details of the development of inbred line NP2052 are as follows:

1988 Stanton, MN Pioneer Brand 3751 was self-pollinated to produce  $S_0$  seed.

1988 Kauai, Hawaii  $S_0$  plants were self pollinated to produce the  $S_1$  generation. Two hundred fourteen  $S_1$  ears were selected from individual  $S_0$  plants based upon plant quality, root strength, ear size, and resistance to diseases.

1989 Stanton, MN Ears of the two hundred fourteen selected  $S_1$  families were grown, observed, and self-pollinated to produce the  $S_2$  generation. Phenotypic selection of these  $S_1$  families was based upon plant quality, synchrony of pollen shed and silk emergence, root strength, ear size, and resistance to disease. Testcross pollinations of the  $S_1$  families were also made.

1990 Stanton, MN Ear rows of the selected  $S_2$  families were grown and observed. The  $S_2$  families were also self-pollinated to produce the  $S_3$  generation. Selection of the  $S_2$  families was based upon the performance of the  $S_1$  testcrosses for grain yield, maturity, and general quality. These testcrosses were grown at several locations. Phenotypic selection of the  $S_2$  families was based upon plant quality, synchrony of pollen shed and silk emergence, root strength, ear size, and resistance to disease. Testcrosses of  $S_2$  families were also made.

1990 Puerto Rico Ear rows of selected  $S_3$  families were grown, observed, and self-pollinated to produce the  $S_4$  generation. Selection of  $S_3$  families was based upon performance of the  $S_2$  testcrosses for grain yield, maturity and general quality. These testcrosses were grown at several locations. Phenotypic selection of  $S_3$  families was based upon plant quality, synchrony of pollen shed

and silk emergence, root strength, ear size, and resistance to disease. Testcrosses of the  $S_3$  families were also made.

1991 Stanton, MN Ear rows of selected  $S_4$  families were grown, observed, and self-pollinated to produce the  $S_5$  generation. Selection of the  $S_4$  families was based upon performance of the  $S_2$  and  $S_3$  testcrosses for grain yield, maturity and general quality. These testcrosses were grown at several locations. Phenotypic selection of the  $S_4$  families was based upon synchrony of pollen shed and silk emergence, root strength, ear size, and resistance to disease. Testcrosses of the  $S_4$  families were also made.

1991 Kauai, Hawaii Ear rows of selected  $S_5$  families were grown, observed, and self-pollinated to produce the  $S_6$  generation.

1992 Stanton, MN Ear rows of each selected  $S_6$  family were grown, observed and self-pollinated to produce individual  $S_7$  ears. Selection of the  $S_6$  families was based upon the performance of the  $S_4$  testcrosses for grain yield, maturity, and general quality. These testcrosses were grown at several locations. Phenotypic selection of the  $S_6$  families was based upon synchrony of pollen shed and silk emergence, root strength, ear size, and resistance to disease.

1993 Stanton, MN Rows of each selected  $S_7$  individual ear culture were grown, observed, and self-pollinated to produce "breeder's seed." These families were closely evaluated and selected for uniformity of anther and silk color, plant and ear height, and other characteristics.  $S_8$  individual ears were saved from one  $S_7$  individual ear culture.

1993 Kauai, Hawaii Rows of each selected  $S_8$  individual ear were grown, observed and self-pollinated to produce additional "breeder's seed." Plants were closely evaluated for uniformity of anther and silk color, plant and ear height, and other characteristics. Isozyme testing confirmed the purity of this inbred line.  $S_9$  individual ears were saved from one  $S_8$  individual ear family.

1994 Stanton, MN Rows of the selected  $S_9$  individual ears were grown, observed, and self-pollinated to produce additional "breeder's seed." Plants were closely evaluated for uniformity of anther and silk color, plant and ear height, and other characteristics. Isozyme testing confirmed the purity of this inbred line.

From 1994 to the present this inbred line has been observed in Stanton, MN and other locations. No phenotypic or isozymic variants have been observed. The variety NP2052 has been uniform and stable.

The invention also encompasses plants of inbred maize line NP2052 and parts thereof further comprising one or more specific, single gene traits which have been introgressed into inbred maize line NP2052 from another maize line. Preferably, one or more new traits are transferred to inbred maize line NP2052, or, alternatively, one or more traits of inbred maize line NP2052 are altered or

substituted. The transfer (or introgression) of the trait(s) into inbred maize line NP2052 is for example achieved by recurrent selection breeding, for example by backcrossing. In this case, inbred maize line NP2052 (the recurrent parent) is first crossed to a donor inbred (the non-recurrent parent) that carries the appropriate gene(s) for the trait(s) in question. The progeny of this cross is then mated back to the recurrent parent followed by selection in the resultant progeny for the desired trait(s) to be transferred from the non-recurrent parent. After three, preferably four, more preferably five or more generations of backcrosses with the recurrent parent with selection for the desired trait(s), the progeny will be heterozygous for loci controlling the trait(s) being transferred, but will be like the recurrent parent for most or almost all other genes (see, for example, Poehlman & Sleper (1995) *Breeding Field Crops*, 4th Ed., 172–175; Fehr (1987) *Principles of Cultivar Development*, Vol. 1: Theory and Technique, 360–376).

The laboratory-based techniques described above, in particular RFLP and SSR, are routinely used in such backcrosses to identify the progenies having the highest degree of genetic identity with the recurrent parent. This permits to accelerate the production of inbred maize lines having at least 90%, preferably at least 95%, more preferably at least 99% genetic identity with the recurrent parent, yet more preferably genetically identical to the recurrent parent, and further comprising the trait(s) introgressed from the donor parent. Such determination of genetic identity is based on molecular markers used in the laboratory-based techniques described above. Such molecular markers are for example those known in the art and described in Boppenmaier, et al., "Comparisons among strains of inbreds for RFLPs", *Maize Genetics Cooperative Newsletter* (1991) 65, pg. 90, or those available from the University of Missouri database and the Brookhaven laboratory database (see <http://www.agron.missouri.edu>). The last backcross generation is then selfed to give pure breeding progeny for the gene(s) being transferred. The resulting plants have essentially all of the morphological and physiological characteristics of inbred maize line NP2052, in addition to the single gene trait(s) transferred to the inbred. Preferably, the resulting plants have all of the morphological and physiological characteristics of inbred maize line NP2052, in addition to the single gene trait(s) transferred to the inbred. The exact backcrossing protocol will depend on the trait being altered to determine an appropriate testing protocol. Although backcrossing methods are simplified when the trait being transferred is a dominant allele, a recessive allele may also be transferred. In this instance it may be necessary to introduce a test of the progeny to determine if the desired trait has been successfully transferred.

Many traits have been identified that are not regularly selected for in the development of a new inbred but that can be improved by backcrossing techniques or genetic transformation. Examples of traits transferred to inbred maize line NP2052 include, but are not limited to, waxy starch,

herbicide tolerance, resistance for bacterial, fungal, or viral disease, insect resistance, enhanced nutritional quality, improved performance in an industrial process, altered reproductive capability, such as male sterility or male fertility, yield stability and yield enhancement. Other traits transferred to inbred maize line NP2052 are for the production of commercially valuable enzymes or metabolites in plants of inbred maize line NP2052.

Traits transferred to maize inbred line NP2052 are naturally occurring maize traits, which are preferably introgressed into inbred maize line NP2052 by breeding methods such as backcrossing, or are heterologous transgenes, which are preferably first introduced into a maize line by genetic transformation using genetic engineering and transformation techniques well known in the art, and then introgressed into inbred line NP2052. Alternatively a heterologous trait is directly introduced into inbred maize line NP2052 by genetic transformation. Heterologous, as used herein, means of different natural origin or represents a non-natural state. For example, if a host cell is transformed with a nucleotide sequence derived from another organism, particularly from another species, that nucleotide sequence is heterologous with respect to that host cell and also with respect to descendants of the host cell which carry that gene. Similarly, heterologous refers to a nucleotide sequence derived from and inserted into the same natural, original cell type, but which is present in a non-natural state, e.g. a different copy number, or under the control of different regulatory sequences. A transforming nucleotide sequence may comprise a heterologous coding sequence, or heterologous regulatory sequences. Alternatively, the transforming nucleotide sequence may be completely heterologous or may comprise any possible combination of heterologous and endogenous nucleic acid sequences.

A transgene introgressed into maize inbred line NP2052 typically comprises a nucleotide sequence whose expression is responsible or contributes to the trait under the control of a promoter appropriate for the expression of the nucleotide sequence at the desired time in the desired tissue or part of the plant. Constitutive or inducible promoters are used. The transgene may also comprise other regulatory elements such as for example translation enhancers or termination signals. In a preferred embodiment, the nucleotide sequence is the coding sequence of a gene and is transcribed and translated into a protein. In another preferred embodiment, the nucleotide sequence encodes an antisense RNA, a sense RNA that is not translated or only partially translated, a t-RNA, a r-RNA or a sn-RNA.

Where more than one trait are introgressed into inbred maize line NP2052, it is preferred that the specific genes are all located at the same genomic locus in the donor, non-recurrent parent, preferably, in the case of transgenes, as part of a single DNA construct integrated into the donor's genome. Alternatively, if the genes are located at different genomic loci in the donor, non-recurrent

parent, backcrossing allows to recover all of the morphological and physiological characteristics of inbred maize line NP2052 in addition to the multiple genes in the resulting maize inbred line.

The genes responsible for a specific, single gene trait are generally inherited through the nucleus. Known exceptions are, e.g. the genes for male sterility, some of which are inherited cytoplasmically, but still act as single gene traits. In a preferred embodiment, a heterologous transgene to be transferred to maize inbred line NP2052 is integrated into the nuclear genome of the donor, non-recurrent parent. In another preferred embodiment, a heterologous transgene to be transferred to maize inbred line NP2052 is integrated into the plastid genome of the donor, non-recurrent parent. In a preferred embodiment, a plastid transgene comprises one gene transcribed from a single promoter or two or more genes transcribed from a single promoter.

In a preferred embodiment, a transgene whose expression results or contributes to a desired trait to be transferred to maize inbred line NP2052 comprises a virus resistance trait such as, for example, a MDMV strain B coat protein gene whose expression confers resistance to mixed infections of maize dwarf mosaic virus and maize chlorotic mottle virus in transgenic maize plants (Murry et al. *Biotechnology* (1993) 11:1559-64). In another preferred embodiment, a transgene comprises a gene encoding an insecticidal protein, such as, for example, a crystal protein of *Bacillus thuringiensis* or a vegetative insecticidal protein from *Bacillus cereus*, such as VIP3 (see for example Estruch et al. *Nat Biotechnol* (1997) 15:137-41). In a preferred embodiment, an insecticidal gene introduced into maize inbred line NP2052 is a Cry1Ab gene or a portion thereof, for example introgressed into maize inbred line NP2052 from a maize line comprising a Bt-11 event as described in U.S. Patent No. 6,114,608, which is incorporated herein by reference, or from a maize line comprising a 176 event as described in Koziel *et al.* (1993) *Biotechnology* 11: 194-200. In yet another preferred embodiment, a transgene introgressed into maize inbred line NP2052 comprises a herbicide tolerance gene. For example, expression of an altered acetohydroxyacid synthase (AHAS) enzyme confers upon plants tolerance to various imidazolinone or sulfonamide herbicides (U.S. Patent No. 4,761,373, incorporated herein by reference). In another preferred embodiment, a non-transgenic trait conferring tolerance to imidazolinones is introgressed into maize inbred line NP2052 (e.g a "IT" or "IR" trait). U.S. Patent No. 4,975,374, incorporated herein by reference, relates to plant cells and plants containing a gene encoding a mutant glutamine synthetase (GS) resistant to inhibition by herbicides that are known to inhibit GS, e.g. phosphinothricin and methionine sulfoximine. Also, expression of a *Streptomyces bar* gene encoding a phosphinothricin acetyl transferase in maize plants results in tolerance to the herbicide phosphinothricin or glufosinate (U.S. Patent No. 5,489,520, incorporated herein by reference). U.S. Patent No. 5,013,659, incorporated herein by reference, is directed to plants that express a mutant acetolactate synthase (ALS) that renders the plants resistant to

inhibition by sulfonyleurea herbicides. U.S. Patent No. 5,162,602, incorporated herein by reference, discloses plants tolerant to inhibition by cyclohexanedione and aryloxyphenoxypropanoic acid herbicides. The tolerance is conferred by an altered acetyl coenzyme A carboxylase (ACCase). U.S. Patent No. 5,554,798, incorporated herein by reference, discloses transgenic glyphosate tolerant maize plants, which tolerance is conferred by an altered 5-enolpyruvyl-3-phosphoshikimate (EPSP) synthase gene. U.S. Patent No. 5,804,425, incorporated herein by reference, discloses transgenic glyphosate tolerant maize plants, which tolerance is conferred by an EPSP synthase gene derived from *Agrobacterium tumefaciens* CP-4 strain. Also, tolerance to a protoporphyrinogen oxidase inhibitor is achieved by expression of a tolerant protoporphyrinogen oxidase enzyme in plants (U.S. Patent No. 5,767,373, which is incorporated herein by reference). Another trait transferred to inbred maize line NP2052 confers tolerance to an inhibitor of the enzyme hydroxyphenylpyruvate dioxygenase (HPPD) and transgenes conferring such trait are, for example, described in WO 9638567, WO 9802562, WO 9923886, WO 9925842, WO 9749816, WO 9804685 and WO 9904021.

In a preferred embodiment, a transgene transferred to maize inbred line NP2052 comprises a gene conferring tolerance to a herbicide and at least another nucleotide sequence encoding another trait, such as for example, an insecticidal protein. Such combination of single gene traits is for example a Cry1Ab gene and a *bar* gene.

Specific transgenic events introgressed into maize inbred line NP2052 are found at [http://www.aphis.usda.gov/bbep/bp/not\\_reg.html](http://www.aphis.usda.gov/bbep/bp/not_reg.html). For example, introgressed from glyphosate tolerant event GA21 (9709901p), glyphosate tolerant/Lepidopteran insect resistant event MON 802 (9631701p), Lepidopteran insect resistant event DBT418 (9629101p), male sterile event MS3 (9522801p), Lepidopteran insect resistant event Bt11 (9519501p), phosphinothricin tolerant event B16 (9514501p), Lepidopteran insect resistant event MON 80100 (9509301p), phosphinothricin tolerant events T14, T25 (9435701p), Lepidopteran insect resistant event 176 (9431901p).

The introgression of a Bt11 event into a maize line, such as maize inbred line NP2052, by backcrossing is exemplified in U.S. Patent No. 6,114,608, and the present invention is directed to methods of introgressing a Bt11 event into maize inbred line NP2052 using for example the markers described in U.S. Pat. No. 6,114,608 and to resulting maize lines.

Direct selection may be applied where the trait acts as a dominant trait. An example of a dominant trait is herbicide tolerance. For this selection process, the progeny of the initial cross are sprayed with the herbicide prior to the backcrossing. The spraying eliminates any plant which do not have the desired herbicide tolerance characteristic, and only those plants that have the herbicide tolerance gene are used in the subsequent backcross. This process is then repeated for the additional backcross generations.

This invention also is directed to methods for producing a maize plant by crossing a first parent maize plant with a second parent maize plant wherein either the first or second parent maize plant is a maize plant of inbred line NP2052 or a maize plant of inbred line NP2052 further comprising one or more single gene traits. Further, both first and second parent maize plants can come from the inbred maize line NP2052 or an inbred maize plant of NP2052 further comprising one or more single gene traits. Thus, any such methods using the inbred maize line NP2052 or an inbred maize plant of NP2052 further comprising one or more single gene traits are part of this invention: selfing, backcrosses, hybrid production, crosses to populations, and the like. All plants produced using inbred maize line NP2052 or inbred maize plants of NP2052 further comprising one or more single gene traits as a parent are within the scope of this invention. Advantageously, inbred maize line NP2052 or inbred maize plants of NP2052 further comprising one or more single gene traits are used in crosses with other, different, maize inbreds to produce first generation (F1) maize hybrid seeds and plants with superior characteristics.

In a preferred embodiment, seeds of inbred maize line NP2052 or seeds of inbred maize plants of NP2052 further comprising one or more single gene traits are provided as an essentially homogeneous population of inbred corn seeds. Essentially homogeneous populations of inbred seed are those that consist essentially of the particular inbred seed, and are generally purified free from substantial numbers of other seed, so that the inbred seed forms between about 90% and about 100% of the total seed, and preferably, between about 95% and about 100% of the total seed. Most preferably, an essentially homogeneous population of inbred corn seed will contain between about 98.5%, 99%, 99.5% and about 100% of inbred seed, as measured by seed grow outs. The population of inbred corn seeds of the invention is further particularly defined as being essentially free from hybrid seed. The inbred seed population may be separately grown to provide an essentially homogeneous population of plants of inbred maize line NP2052 or inbred maize plants of NP2052 further comprising one or more single gene traits.

As used herein, the term "plant" includes plant cells, plant protoplasts, plant cell tissue cultures from which maize plants can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of plants, such as embryos, pollen, ovules, flowers, kernels, ears, cobs, leaves, husks, stalks, roots, root tips, anthers, silk, seeds and the like.

Duncan, Williams, Zehr, and Widholm, *Planta* (1985) 165:322-332 reflects that 97% of the plants cultured that produced callus were capable of plant regeneration. Subsequent experiments with both inbreds and hybrids produced 91% regenerable callus that produced plants. In a further study in 1988, Songstad, Duncan & Widholm in *Plant Cell Reports* (1988), 7:262-265 reports several media additions that enhance regenerability of callus of two inbred lines. Other published reports also



Tissue culture procedures of maize are described in Green and Rhodes, "Plant Regeneration in Tissue Culture of Maize," Maize for Biological Research (Plant Molecular Biology Association, Charlottesville, Va. 1982, at 367-372) and in Duncan, et al., "The Production of Callus Capable of Plant Regeneration from Immature Embryos of Numerous Zea mays Genotypes," 165 Planta 322-332 (1985). Thus, another aspect of this invention is to provide cells that upon growth and differentiation produce maize plants having the physiological and morphological characteristics of inbred maize line NP2052. In a preferred embodiment, cells of inbred maize line NP2052 are transformed genetically, for example with one or more genes described above, for example by using a transformation method described in U.S. Pat. No. 6,114,608, and transgenic plants of inbred maize line NP2052 are obtained and used for the production of hybrid maize plants.

Maize, including both grain and non-grain portions of the plant, is also used extensively as livestock feed, primarily for beef cattle, dairy cattle, hogs, and poultry. Industrial uses of maize include production of ethanol, maize starch in the wet-milling industry and maize flour in the dry-milling industry. The industrial applications of maize starch and flour are based on functional properties, such as viscosity, film formation, adhesive properties, and ability to suspend particles. The maize starch and flour have application in the paper and textile industries. Other industrial uses include applications in adhesives, building materials, foundry binders, laundry starches, explosives, oil-well muds, and other mining applications. Plant parts other than the grain of maize are also used in industry: for example, stalks and husks are made into paper and wallboard and cobs are used for fuel and to make charcoal.

22

produced from the crossing of the inbred, hybrid seed, and various parts of the hybrid maize plant can be utilized for human food, livestock feed, and as a raw material in industry.

The present invention therefore also discloses an agricultural product comprising a plant of the present invention or derived from a plant of the present invention. The present invention also discloses an industrial product comprising a plant of the present invention or derived from a plant of the present invention. The present invention further discloses methods of producing an agricultural or industrial product comprising planting seeds of the present invention, growing plant from such seeds, harvesting the plants and processing them to obtain an agricultural or industrial product.

#### PERFORMANCE EXAMPLES OF MAIZE INBRED LINE NP2052

In the examples that follow, the traits and characteristics of inbred maize line NP2052 are presented as an comparison between it and inbred line A619. The data presented is for key characteristics and traits and is reported from experiments where the compared lines were grown side-by-side.

The data in Table 2 show, for example, that the pollen shed of variety NP2052 is significantly shorter than A619. The length of shed for NP2052 is 74 heat units as compared to 94 for A619. Table 2 also shows that the length of the top ear internode is significantly shorter for NP2052 compared to A619, and that the length of the ear node leaf of NP2052 is significantly longer than A619.

TABLE 2

	H.U. 10% to 90% Pollen Shed	Length of Top Ear Internode (cm)	Length of Ear Node Leaf (cm)	Leaf Sheath Pubescence (1-9 rating)
Grand Mean	84	11	63	3
Trails w/data	5	6	5	6
LSD (= 95% confidence level)	16	1	3	2
CV%	27	12	4	35
Probability %	2	0	0	1
A619	94	12	61	2
NP2052	74	10	65	5

TABLE 3  
VARIETY COMPARISON

	STDP	LRTL	ERTLN	ERTLP	GRSN	GRSNP	DROPN
Grand Me	100	20	0	0	0	0	0
Trials w/da	6	3	2	2	2	2	2
LSD	0	161	0	0	0	0	
CV%	0	228					
Probability		42					
A619	100	39	0	0	0	0	0
NP2052	100	1	0	0	0	0	0
	PLHCN	ERHCN	HU1SN	HU5SN	HU9SN	HUPSN	STGRP
Grand Me	171	44	1259	1314	1313	1283	6
Trials w/da	3	3	3	3	3	3	3
LSD	46	15	9	354	494	12	0
CV%	6	29	1	2	2	1	0
Probability	67	49	0	73	51	0	
A619	168	41	1227	1308	1296	1257	6
NP2052	174	46	1305	1322	1338	1322	7
	HUPLN	PLQUR	EARPN	SHLNN	HSKCR	OTHIN	
Grand Me	35	6	1	8	3	0	
Trials w/da	3	3	3	3	2	3	
LSD	22	1	0	3	6	0	
CV%	65	22	22	33	0		
Probability	0	9	17	99	20		
A619	55	7	1	8	3	0	
NP2052	15	6	1	8	4	0	
	EINDN	LTEIN	ERLWN	ERLLN	LAERN	ANGBN	LTBRN
Grand Me	5	11	9	71	4	41	8
Trials w/da	4	3	3	3	3	3	3
LSD		6	2	3	7	8	141
CV%		12	20	3	14	18	21
Probability		31	77	0	71	16	49
A619	2	12	10	69	5	38	41
NP2052	3	10	9	73	4	44	69
	TBANN	LTASN	ERLNN	ERDIN	EWGTN	KRRWN	KRLNN
Grand Me	55	39	12	37	79	13	11
Trials w/da	3	3	3	3	3	3	3
LSD	141	2	1	4	14	2	1
CV%	21	5	8	9	16	10	5
Probability	49	77	6	2	0	69	6
A619	41	39	13	39	89	13	11
NP2052	69	39	11	34	68	13	10



What is claimed is:

1. Seed of maize inbred line NP2052 having been deposited under ATCC Accession No: \_\_\_\_\_.
2. A maize plant, or parts thereof, of inbred line NP2052, seed of said line having been deposited under ATCC Accession No: \_\_\_\_\_.
3. Pollen of the plant of claim 2.
4. An ovule of the plant of claim 2.
5. A maize plant, or parts thereof, having all the physiological and morphological characteristics of a plant according to claim 2.
6. A male sterile maize plant, or parts thereof, otherwise having all the physiological and morphological characteristics of a plant according to claim 2.
7. A maize plant, or parts thereof, according to claim 2, further comprising one or more single gene transferred traits.
8. A maize plant, or parts thereof, according to claim 7, wherein the plant or parts thereof have been transformed so that its genetic material contains one or more transgenes operably linked to one or more regulatory elements.
9. A maize plant according to claim 7, wherein said single gene transferred trait comprises a gene conferring upon said maize plant tolerance to a herbicide.
10. A maize plant according to claim 9, wherein said herbicide is glyphosate, gluphosinate, a sulfonylurea or an imidazolinone herbicide, a hydroxyphenylpyruvate dioxygenase inhibitor or a protoporphyrinogen oxidase inhibitor.
11. A maize plant according to claim 7, wherein said single gene transferred trait comprises a gene conferring upon said maize plant insect resistance, disease resistance or virus resistance.

12. A maize plant according to claim 11, wherein said gene conferring upon said maize plant insect resistance is a *Bacillus thuringiensis* Cry1Ab gene.
13. A maize plant according to claim 12, further comprising a *bar* gene.
14. A maize plant according to claim 12, wherein said Cry1Ab gene is introgressed into said maize plant from a maize line comprising a Bt-11 event or a 176 event.
15. Seed of a plant according to claim 7.
16. A tissue culture of regenerable cells of a maize plant according to claim 2, wherein the tissue regenerates plants capable of expressing all the morphological and physiological characteristics of plants according to claim 2.
17. A tissue culture according to claim 16, the regenerable cells being selected from the group consisting of embryos, meristems, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks and stalks, or being protoplasts or callus derived therefrom.
18. A maize plant regenerated from the tissue culture of claim 16, capable of expressing all the morphological and physiological characteristics of inbred line NP2052, seed of said inbred line having been deposited under ATCC Accession No: \_\_\_\_\_.
19. A method for producing maize seed comprising crossing a first parent maize plant with a second parent maize plant and harvesting the resultant first generation maize seed, wherein said first or second parent maize plant is the inbred maize plant of claim 2.
20. A method according to claim 19, wherein said first parent maize plant is different from said second parent maize plant, wherein said resultant seed is a first generation (F1) hybrid maize seed.
21. A method according to claim 19, wherein inbred maize plant of claim 2 is the female parent.
22. A method according to claim 19, wherein inbred maize plant of claim 2 is the male parent.

- 28

37. A method comprising:

(a) planting a collection of seed comprising seed of a hybrid, one of whose parents is a plant according to claim 2, or a maize plant having all the physiological and morphological characteristics of a plant according to claim 2, said collection also comprising seed of said inbred line;

(b) growing plants from said collection of seed;

(c) identifying said inbred plants;

(d) selecting said inbred plant; and

(e) controlling pollination in a manner which preserves the homozygosity of said inbred plant.

38. A method according to claim 37, wherein said one parent is a plant of inbred maize line NP2052, further comprising a single gene transferred trait.

39. The method of claim 37, wherein said step of identifying said inbred plant comprises identifying plants with decreased vigor.

40. A method comprising introgressing a single gene trait into inbred maize line NP2052, seed of said line having been deposited under ATCC Accession No: \_\_\_\_\_, using one or more markers for marker assisted selection among maize lines to be used in a maize breeding program, the markers being associated with a single gene trait, wherein the resulting maize line is inbred maize line NP2052 further comprising said single gene transferred trait.

41. A method according to claim 40, wherein said a single gene trait comprises a Cry1Ab gene.

42. A NP2052-derived maize plant, or parts thereof, wherein at least one ancestor of said maize plant is the maize plant of claim 2, said maize plant expressing a combination of at least two NP2052 traits selected from the group consisting of: a relative maturity of approximately 85 to 105 days based on the Comparative Relative Maturity Rating System for harvest moisture of grain, acceptable to good grain quality, good Northern Corn Leaf Blight resistance, good Eyespot resistance, good Common Rust resistance, good First Brood Corn Borer resistance, above average early growth, good seedling vigor, early pollen shed, resistance to stalk diseases, reliable late season plant health, average pollen shed, improved stalk strength, acceptable late season intactness, and adapted to the Northern Cornbelt regions of the United States.



## ABSTRACT

An inbred maize line, designated NP2052, the plants and seeds of inbred maize line NP2052 and descendants thereof, methods for producing a maize plant produced by crossing the inbred line NP2052 with itself or with another maize plant, and hybrid maize seeds and plants produced by crossing the inbred line NP2052 with another maize line or plant.

00707589-110700

DECLARATION AND POWER OF ATTORNEY FOR U.S. PATENT APPLICATIONS

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,  
and

I believe I am the original, first and sole inventor of the subject matter which is claimed  
and for which a patent is sought on the invention entitled

INBRED MAIZE LINE NP2052

the specification of which was filed on **TBA** as U.S. Application No. **TBA**.

I hereby state that I have reviewed and understand the contents of the above identified  
specification, including the claims.

I acknowledge my duty to disclose all information which is known by me to be material  
to the patentability of this application as defined in 37 C.F.R. §1.56.

I hereby claim the benefit under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign  
application(s) for patent or inventor's certificate listed below and under 35 U.S.C. §365(a) of any  
PCT international application(s) designating at least one country other than the United States  
listed below and have also listed below any foreign application(s) for patent or inventor's  
certificate or any PCT international application(s) designating at least one country other than  
the United States for the same subject matter and having a filing date before that of the  
application the priority of which is claimed for that subject matter:

None

I hereby claim the benefit under 35 USC §119(e) of any United States provisional  
application(s) listed below:

None

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s)  
listed below and under 35 U.S.C. §365(c) of any PCT international application(s) designating

00207T 68520260

the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in said prior application(s) in the manner required by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose all information known by me to be material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date(s) of the prior application(s) and the national or PCT international filing date of this application:

None

I hereby appoint the attorneys and agents associated with Customer No. 022847, respectively and individually, as my attorneys and agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Please address all communications to the address associated with Customer No. 022847, which is currently Larry W. Stults, Novartis Agribusiness Biotechnology Research Inc., Patent Department, P.O. Box 12257, Research Triangle Park, NC 27709-2257.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SOLE INVENTOR:

Full name : **Christopher Perry**

Signature : \_\_\_\_\_

Date : \_\_\_\_\_  
(MM/DD/YY)

Citizenship : United States of America

Residence : Northfield, Minnesota

P.O. Address : 731 Birch Lane  
Northfield, Minnesota 55057

IMPORTANT: Before this declaration is signed, the patent application (the specification, the claims and this declaration) must be read and understood by each person signing it, and no changes may be made in the application after this declaration has been signed.

09707589-110700